Synthesis and Antimicrobial Evaluation of Amphiphilic Neamine Derivatives

Isabelle Baussanne,[†] Antoine Bussière,[†] Somnath Halder,[†] Carine Ganem-Elbaz,[‡] Myriam Ouberai,[§] Mickael Riou,[§] Jean-Marc Paris,[‡] Eric Ennifar,[||] Marie-Paule Mingeot-Leclercq,[§] and Jean-Luc Décout*,[†]

[†]*Université de Grenoble I/CNRS, UMR 5063, Département de Pharmacochimie Moléculaire, ICMG FR 2607, France,* [‡]*Ecole Nationale Supérieure de Paris/CNRS, UMR 7573, France,* [§]*Université Catholique de Louvain, Louvain Drug Research Institute, LDRI, Unité de Pharmacologie Cellulaire et Moléculaire, FACM, Brussels, Belgium, and* [||]*Université de Strasbourg/CNRS, Institut de Biologie Moléculaire et Cellulaire, Architecture et Réactivité de l'ARN, France*

The aminoglycoside antibiotics bind to the 16S bacterial rRNA and disturb the protein synthesis. One to four hydroxyl functions of the small aminoglycoside neamine were capped with phenyl, naphthyl, pyridyl, or quinolyl rings. The 3′,4′- (**6**), 3′,6- (**7a**), and the 3′,4′,6- (**10a**) 2-naphthylmethylene derivatives appeared to be active against sensitive and resistant *Staphylococcus aureus* strains. **10a** also showed marked antibacterial activities against Gram (−) bacteria, including strains expressing enzymes modifying aminoglycosides, efflux pumps, or rRNA methylases. **7a** and **10a** revealed a weak and aspecific binding to a model bacterial 16S rRNA. Moreover, as compared to neomycin B, **10a** showed a lower ability to decrease ^{3}H leucine incorporation into proteins in *Pseudomonas aeruginosa*. All together, our results suggest that the 3′,4′,6-tri-2-naphthylmethylene neamine derivative **10a** should act against Gram (−) bacteria through a mechanism different from inhibition of protein synthesis, probably by membrane destabilization.

Introduction

Ribosomal RNA (rRNA) is the main target of clinically important antibiotics such as the natural drugs aminoglycosides. These antibacterial agents are active against both Gram (−) and Gram (+) pathogens and continue to play an important role to treat serious infections, usually in combination with *β*-lactams.[1] The aminoglycosides are pseudo-oligosaccharides, which carry generally four to six amine functions positively charged at pH 7. They bind to the A-site of the 16S bacterial rRNA, cause mRNA decoding errors, block mRNA and tRNA translocation, and inhibit ribosome recycling.[2] Neomycin B is one of the most studied aminoglycosides.[1,2] As observed with other aminoglycosides, a high level of resistance to neomycin B that involves enzymatic modifications of the hydroxyl and amine functions has emerged.[3] Moreover, since 2003, methylation of 16S rRNA appeared to be a serious threat to the aminoglycoside antibiotics through the action of plasmid-mediated methyltransferases.[4] These enzymes are spreading to different species and are found to confer high levels of resistance to clinically useful aminoglycosides like amikacin, tobramycin, and gentamicin. Methylation of specific nucleotides within the A-site of rRNA hampers binding of aminoglycosides.

In the search for new antibiotic agents, neamine **1** (Figure 1) incorporating two rings of neomycin B has appeared to be the minimum scaffold necessary for binding to 16S rRNA[5] as well as an attractive starting molecule.[6] Earlier, neamine derivatives have been prepared through modifications of the amino

groups or the 3′-, 5-, or 6-hydroxyl functions in order to increase the affinity for rRNA and prevent the activity of aminoglycoside-modifying enzymes.[6]
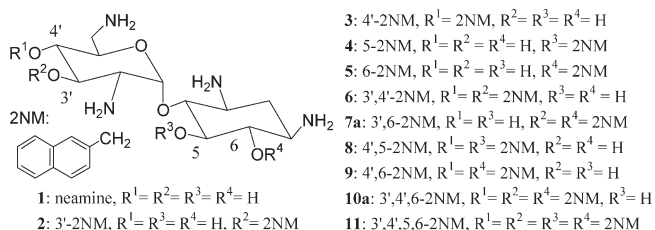
To further improve the activity of neamine derivatives and protect them from enzymatic modifications, we introduced aromatic rings from the hydroxyl functions of neamine through reactions with arylmethylene bromides. These modifications, leading to more lipophilic compounds than neamine, should increase the affinity and the specificity for different RNA targets through groove binding and/or stacking. In this approach, the naphthyl ring appeared attractive. Indeed, the affinity of polyamines consisting of ethylenediamine units equipped with either one or two naphthyl, anthryl, or acridyl units toward polyA.polyU, as a RNA model, and poly(dA).poly(dT), as a DNA model, has been reported.[7] A higher selectivity for binding to RNA in comparison to DNA has been observed with naphthyl derivatives, the larger aromatic units preferring binding to DNA. This difference was explained through a preference for groove binding of the naphthyl rings and for intercalation in DNA of the larger aromatic rings.

To establish the rational basis of structure/activity relationships, we synthesized sixteen mono-, di-, tri-, and tetra-arylmethylene neamine derivatives and determined their antibacterial activity on Gram (+) and Gram (−) bacteria, sensitive, and resistant strains.

Results

Chemistry. Previously, we have reported a route for preparing 4′-, 5-, and 4′,5-neamine derivatives using trityl (Tr) and *p*-methoxybenzyl (PMB) groups for protecting the amine and hydroxyl functions, respectively.[8]

*To whom correspondence should be addressed. Phone: +33476635317. Fax: +33476635298. E-mail: Jean-Luc.Decout@ujf-grenoble.fr.

3: 4'-2NM, R^1= 2NM, R^2= R^3= R^4= H
4: 5-2NM, R^1= R^2= R^4= H, R^3= 2NM
5: 6-2NM, R^1= R^2= R^3= H, R^4= 2NM
6: 3',4'-2NM, R^1= R^2= 2NM, R^3= R^4= H
7a: 3',6-2NM, R^1= R^3= H, R^2= R^4= 2NM
8: 4',5-2NM, R^1= R^3= 2NM, R^2= R^4= H
9: 4',6-2NM, R^1= R^4= 2NM, R^2= R^3= H
10a: 3',4',6-2NM, R^1= R^2= R^4= 2NM, R^3= H
11: 3',4',5,6-2NM, R^1= R^2= R^3= R^4= 2NM

1: neamine, R^1= R^2= R^3= R^4= H
2: 3'-2NM, R^1= R^3= R^4= H, R^2= 2NM

Figure 1. Structure of the prepared 2-naphthylmethylene (2NM) neamine derivatives.

Scheme 1. General Method Used for the Preparation of the 3',6-Di- (**7**) and 3',4',6-Trialkylated (**10**) Neamine Derivatives

a, R= 2NM: b, R= Bn   c, R= 2PM: d, R= 2QM:
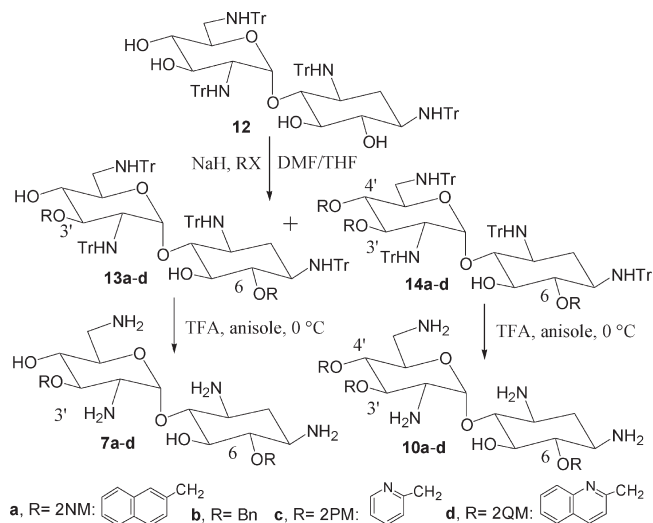
The 4'-mono-2-naphthylmethylene (4'-2NM,[a] **3**), the 5-2NM (**4**), and the 4',5-2NM (**8**) derivative (Figure 1) were synthesized from compound **12** (Scheme 1) according to the methodology previously described.

The difference in the stability of the *O*-2NM and the *N*-trityl groups under acidic conditions allowed the preparation of the 3',6-2NM (**7a**) and the 3',4',6-2NM (**10a**) derivatives from **12** in two steps: (i) alkylation of the hydroxyl fonctions and (ii) selective removal of the trityl groups under acidic conditions (Scheme 1). The reaction of **12** with 2-bromomethylnaphthalene (2NMBr) led to a mixture (1:1) of the tritylated 3',6-2NM (**13a**) and 3',4',6-2NM (**14a**) derivatives in 40% and 38% yields, respectively. Their treatment with TFA/anisole at 0 °C allowed the selective detritylation leading to **7a** (70%) and **10a** (65%), which appeared soluble in water.

The 3',4',5,6-2NM derivative **11** (Figure 1) was prepared by alkylation of **12** with 2NMBr in excess and removal of the trityl groups (40%). Two other di2NM derivatives, the 3',4'-2NM (**6**) and 4',6-2NM (**9**) isomers, were prepared from **14a** and **12**, respectively. The instability of the 2NM ethers in TFA and the highest reactivity of the 6-alcoholate function of **12** observed in polar solvents suggested a high sensitivity of the 6-*O*-2NM group to TFA.[9] Therefore, it could be

possible to prepare **6** from **14a** through the removal under acidic conditions of the trityl and the 6-2NM groups. **14a** was treated with TFA increasing both the temperature (rt) and the reaction time in comparison to the conditions leading to **10a** from **14a**. **6** was isolated as the major product (51%). The 4',6-2NM derivative **9** was prepared from **12** in three steps: (i) protection of the 3'-OH function through reaction with PMB chloride in the presence of NaH in toluene in order to slow down the velocities of alkylation of the alcoholate functions in comparison to the DMF/THF mixture (55%), (ii) 4'- and 6-*O*-alkylations with 2NMBr in DMF, and (iii) removal of the trityl and 3'-PMB groups under acidic conditions (15% for the two last steps). The low yield can be explained by the sensitivity of the 4'- and 6-2NM ether groups to TFA.

The 3'-2NM derivative **2** was obtained as a minor product through the partial deprotection of the tetratrityl intermediate **13a** (22%) under the conditions used previously for the preparation of **6**. To prepare the 6-2NM derivative **5** from **12** and 2NMBr, the proportion of DMF in the DMF/THF mixture previously used for the preparation of **13a** and **14a** from **12** was increased from 1:9 to 1:1.[9] In the presence of NaH, **12** reacted to lead mainly to the tetratrityl 6-2NM derivative isolated in low yield (12%, **12** was principally recovered). The resulting compound was detritylated in TFA at 0 °C to give the 6-2NM derivative **5** (82%).

To evaluate the role of the naphthyl ring in the antibiotic effects, the 2NM groups in **7a** and **10a** were replaced by benzyl (Bn), 2-pyridylmethylene (2PM), or 2-quinolylmethylene (2QM) groups (Scheme 1). The phenyl and pyridyl rings were selected for their weak stacking in nucleic acids. The diBn (**7b**, 34%) and triBn (**10b**, 45%), the di2PM (**7c**, 24%) and tri2PM (**10c**, 3%), and the di2QM (**7d**, 42%) and tri2QM (**10d**, 4%) derivatives were obtained according to the methodology developed for preparing **7a** and **10a**. The low yields in 2PM and 2QM derivatives resulted from the sensitivity of the corresponding ether groups to TFA.

Antibiotic Activity. The neamine derivatives synthesized were evaluated against *Staphylococcus aureus*, sensitive and resistant strains expressing resistance pumps (NorA or MsrA), inactivating enzymes like APH2''-AAC6', APH3', or ANT4' or against MRSA and VRSA resistant to methicillin and vancomycin, respectively (Table 1). Their effects against Gram (−), *Acinetobacter lwoffi, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Escherichia coli* sensitive and resistant strains, expressing aminoglycoside-modifying enzymes, efflux pumps (Table 2), or r-methylase were also determined.

On *S. aureus*, in the 2NM series, the four mono derivatives (**2**, **3**, **4**, **5**) appeared totally inefficient (Table 1). In contrast, the 3',6-2NM (**7a**) and 3',4',6-2NM (**10a**) derivatives were active and for the latter showed remarkable activity against MRSA and VRSA as well as *S. aureus* expressing APH3' and ANT4', toward which neomycin B was inactive. On Gram (−) bacteria, **7a** showed no or only moderate antimicrobial effects (Table 2), whereas **10a** revealed an antibacterial activity (MIC = 4−16 μg/mL) both on Enterobacteriaceae and non-Enterobacteriacea including *P. aeruginosa* expressing efflux pumps (Tables 2 and 3). This derivative was also active [MIC = 4−8 μg/mL] on strains expressing r-methylase (*Citrobacter amalonaticus* arm 06AB0010, *E. coli* 06AB003 arm, *Enterobacter aerogenes* 06AB008 arm) against which gentamicin, amikacin, and tobramycin were totally inactive (MIC > 128 μg/mL). Increasing the number of naphthyl to

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Table 1. Minimum Inhibitory Concentrations against Different *Staphylococcus aureus* Strains for the Neamine Derivatives, Neomycin B, and Neamine

	MIC μg/mL							
aminoglycosides	ATCC 25923	pump NorA	pump MsrA	enzyme APH2''-AAC6'	enzyme APH3'	enzyme ANT4'	ATCC 33592 HA-MRSA	VRSA-VRS-2
neomycin B	2	1	2	1	>128	32	>128	128
neamine **1**	32	32	16	16	>128	>128	>128	>128
3'-mono2NM **2**	>128	>128	>128	>128	128	>128	ND	ND
4'-mono2NM **3**	>128	>128	>128	>128	>128	>128	ND	ND
5-mono2NM **4**	>128	>128	>128	>128	>128	>128	ND	ND
6-mono2NM **5**	>128	>128	>128	>128	>128	>128	>128	ND
3',4'-di2NM **6**	4	8	8	8	4	8	8	4
3',6-di2NM **7a**	8	8	8	8	4	8	16	16
4',5-di2NM **8**	64	128	128	128	32	128	64	64
4',6-di2NM **9**	32	32	32	32	16	16	64	32
3',4',6-tri2NM **10a**	4	4	4	4	2	4	2	4
3',4',5,6-tetra2NM **11**	32	64	64	64	32	64	32	64
3',6-diBn **7b**	>128	>128	>128	>128	>128	>128	ND	ND
3',6-di2PM **7c**	>128	>128	>128	>128	>128	>128	ND	ND
3',6-di2QM **7d**	>128	>128	>128	>128	>128	>128	ND	ND
3',4',6-triBn **10b**	>128	>128	>128	>128	>128	>128	>128	64
3',4',6-triPM **10c**	>128	>128	>128	>128	>128	>128	ND	ND
3',4',6-tri2QM **10d**	128	>128	>128	128	64	>128	64	64

Table 2. Minimum Inhibitory Concentrations against Selected Bacterial Gram (−) Susceptible and Resistant Strains through Enzymatic Modification and Efflux[a]

	MIC μg/mL								
	A. lwoffi		*P. aeruginosa*			*K. pneumoniae*	*E. coli*		
aminoglycosides	a	b	c	d	e	f	g	h	i
gentamicin	0.5	4−8	1	>128	4	8	<0.5−1	1	64−128
amikacin	0.5	>128	2−4	4	8−16	0.5	4	32	2
tobramycin	0.5	1	0.5	128	1	4	0.5	32	64
neomycin B	0.5	>128	64	128	32	16−32	2	4	32
neamine **1**	2	>128	>128	>128	>128	32−64	32	>128	32
3',4'-di2NM **6**	8	>128	32	>128	>128	>128	32	16	16
3',6-di2NM **7**	64	>128	128	128	>128	128− >128	64	64	64
4',5-di2NM **8**	32	>128	128	>128	>128	>128	128	128	128
4',6-di2NM **9**	64	>128	>128	>128	>128	>128	128	64	128
3',4',6-tri2NM **10a**	4	32	8	8	4	16	16	4	4
3',4',5,6-tetra2NM **11**	32	>128	64	64	64	128	128	64	64
3',4',6-triBn **10b**	>128	>128	>128	>128	>128	>128	>128	>128	>128
3',4',6-tri2QM **10d**	128	>128	>128	>128	>128	>128	>128	>128	>128

[a] a: ATCC 17925; b: AI.88-483 APH3'-VIa; c: ATCC 27853; d: Psa.F03 AAC6'-IIa; e: PA22 (PT629) surexp MexXY; f: ATCC 700603; g: ATCC 25922 ; h: PAZ505H8101 AAC6'-IIb; i: L58058.1 ANT2''-IIa.

four with **11** resulted in a strong or complete loss of activity both on Gram (+) (Table 1) and Gram (−) (Tables 2, 3) bacteria.

Regarding the importance of the position of the 2NM groups in the di2NM neamine derivatives, with the 3',4'-2NM derivative **6**, we observed antibacterial effects against the *S. aureus* strains similar to those of the 3',6-isomer **7a** (Table 1). Also like **7a**, **6** appeared inefficient against Gram (−) strains (Table 2). The 4',5-2NM derivative **8** and the 4',6-2NM isomer **9** were less active or inefficient both against *S. aureus* and Gram (−) bacteria (Tables 2 and 3).

The replacement of the naphthyl rings in **7a** and **10a** by benzyl, pyridyl, or quinolyl rings led to the complete disappearance of the antibiotic activities both on Gram (+) and Gram (−) bacteria (**7b−d**: Table 1; **10b,d**: Tables 2 and 3).

Alteration of Protein Synthesis. The ability of **10a** to decrease the incorporation of ^{3}H leucine[10] (Figure 2) was determined in comparison to neamine **1**, neomycin B, and aztreonam, a monolactam antibiotic acting on the cell wall. As expected, both neamine and aztreonam had no effect on synthesis of proteins. In contrast, the inhibition induced by

neomycin B was around 40% at the MIC and reached 95% at 10-fold the MIC. **10a** also decreased the incorporation of radiolabeled leucine, but the concentrations required to obtain a given effect were greater than those required with neomycin B. Ten-fold the MIC led to ∼65% inhibition. The inhibitory profile of **10a** was quite similar to that of polymyxin E (also known as colistin),[11] a bactericidal antibiotic acting on bacterial membrane with which 30 and around 95% of decrease in incorporation were observed at 5- and 10-fold MIC, respectively (Figure 2).

Isothermal Titration Calorimetry (ITC). The binding of compounds **7a** and **10a** to a model bacterial rRNA A site was evaluated using ITC.[12] Only unspecific binding[12] (K_d in the 10 μM range) was observed for each compound, whereas a specific interaction ($K_d = 1.55\,\mu$M) was obtained in the same experimental conditions with neomycin B (see as Supporting Information).[13]

Discussion and Conclusion

Sixteen amphiphilic neamine derivatives carrying one to four arylmethylene groups were prepared and evaluated for

Table 3. Minimum Inhibitory Concentrations against *P. aeruginosa* Susceptible WT and Resistant through Overexpression of Efflux Pump for Selected Natural Aminoglycosides and Neamine Derivatives[a]

aminoglycosides	MIC μg/mL							
	a	b	c	d	e	f	g	h
gentamicin	1	2	1	1	1	4	<0.125	<0.125
amikacin	2−4	2−4	2	2−4	2	8−16	0.5	1
tobramycin	0.5	0.5	0.5	<0.25	0.5	1	<0.25	<0.25
neomycinB	64−128	4	8	4	4	32	2	4
neamine **1**	>128	>128	>128	>128	>128	>128	128	128
3'4'-di2NM **6**	16−32	64	16	8	64	>128	8	8
3',6-di2NM **7a**	128	128	32	4	>128	>128	4	4
4',5-di2NM **8**	>128	>128	>128	>128	>128	>128	16	16
4',6-di2NM **9**	>128	>128	128	16	>128	>128	32	16
3',4',6-tri2NM **10a**	4−8	8−16	4	4	4	4	4	4
3',4',5,6-tetra2NM **11**	64	64	64	64	64	64	64	64
3',4',6-triBn **10b**	>128	>128	>128	>128	>128	>128	64	64
3',4',6-tri2QM **10d**	>128	>128	>128	>128	>128	>128	64	64

[a] a: ATCC 27853 WT; b: PA07 (PAO1) WT; c: PA02 surexp MexCD; d: PA03 surexp MexEF; e: PA21 (MutGr1) surexp MexAB; f: PA22 (PT629) surexp MexXY; g: PA405 (PA0509.5) surexp TriABC and deleted for MexAB, MexCD, MexEF, MexXY; h: PA406 (PA0 1095#1) deleted for MexAB, MexCD, MexEF, MexXY, TriABC.
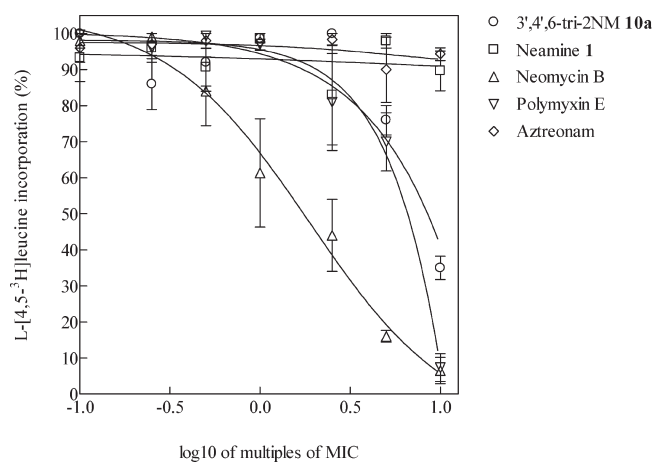
Figure 2. Effects of the 3',4',6-tri2NM neamine derivative **10a**, neamine **1**, neomycin B, polymyxin E, and aztreonam on protein synthesis of *P. aeruginosa* ATCC 27853. L-[4,5-^{3}H]Leucine incorporation into proteins was measured after exposure to 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 times MIC. The ordinate shows the percentage of incorporation of leucine expressed in % of control. Values are mean ($n = 3$) determinations. Error bars represent standard deviations.

their antibacterial properties. Among them, the 3',4'-2NM (**6**), 3',6-2NM (**7a**), and 3',4',6-2NM (**10a**) derivatives showed strong antimicrobial activity against sensitive and resistant *S. aureus*, including MRSA and VRSA toward which neomycin B is totally inactive. When tested against sensitive or resistant Gram (−) organisms, **10a** appeared to be the sole promising agent. It showed MIC values ranging from 4 up to 16 μg/mL against a large panel of sensitive and resistant strains including bacteria expressing rRNA methylases. This feature is particularly exciting because methylation of 16S rRNA is a serious threat to aminoglycosides through the action of plasmid-mediated methyltransferase enzymes and spreading to different species.[4]

The antibacterial effects of the aminoglycosides used as drugs result from their ability to be taken up by the bacteria together with their affinity for rRNA. The conjugation of neamine to 2NM hydrophobic groups leading to more lipophilic compounds than neamine should enhance the penetration of the corresponding derivatives in the phospholipid bilayer of bacteria, resulting in either increased uptake or destabilization of the lipid membrane. The presence of the 2NM groups in the active compounds also should modify the affinity of the neamine core for different RNA sequences through steric hindrance, minor groove binding, and/or stacking.

Regarding potential structure/activity relationships, the four mono2NM derivatives **2**−**5** were inefficient as antibiotic agents and the tetra2NM derivative **11** showed weak antibacterial effects. In contrast, the presence of two or three 2NM groups in the neamine derivatives led to active compounds. On *S. aureus*, among the disubstituted derivatives synthesized, the most active ones are the 3',4'-2NM (**6**) and 3',6-2NM (**7a**) derivatives. Their MICs were much lower than those of the 4',5-2NM (**8**) and 4',6-2NM (**9**) isomers. The replacement of the 2NM moieties in **7a** by benzyl, 2PM, and 2QM groups led to the disappearance of the antimicrobial effects. These data probably exclude a nonspecific detergent effect because the antimicrobial effects observed with the di2NM derivatives depend on the positions of the 2NM groups. Also, a minor change in the structure, i.e., the replacement of the 2NM moieties in **7a** by 2QM groups led to the disappearance of the antimicrobial effects. This conclusion is in accord with previous data obtained with neomycin conjugates to fatty acids, cholesterol, and pyrene.[14] These compounds have shown antibacterial effects against Gram (+) bacteria strictly dependent on the nature of the lipidic moiety.

Only the tri2NM derivative **10a** is active both on Gram (+) and Gram (−) bacteria. The presence of three 2NM groups is necessary for obtaining antimicrobial effects against Gram (−) bacteria. Also, the replacement of the 2NM groups by benzyl, 2PM, or 2QM groups led to the disappearance of the antibiotic effects against both Gram (+) and Gram (−) bacteria, suggesting the critical role of the naphthyl rings. The structure of the substituents and their number appear therefore to be critical parameters.

Several features of the active neamine derivatives prepared should be put forward.

First, the affinity of **7a** and **10a** for a model bacterial ribosomal A site measured by microcalorimetry (ITC) appeared to be weak and aspecific in comparison to the strong binding of neomycin B observed. This result and the low inhibition of leucine incorporation in proteins of *P. aeruginosa* induced by **10a** in comparison to neomycin B (Figure 2) suggest a weak binding of **10a** to rRNA. However, a binding

of **7a** and **10a** to a RNA sequence different from the A site, leading to strong disruption of protein synthesis, remains possible in Gram (+) bacteria.

Second, if the antimicrobial activity of **10a** against Gram (+) and Gram (−) bacteria results, at least in part, from a binding to the bacterial membranes and a corresponding membrane destabilization, the hydrophobic/hydrophilic balance of the active derivative should be a critical parameter for the binding to membranes and lipid bilayer destabilization. This assumption is in agreement with the observed SAR and the critical role of the number of 2NM groups observed.

Third, the loss of activity of the di2NM derivatives **6, 7a, 8** and **9** and the poor activity of the tetra2NM derivative **11** against Gram (−) bacteria could result from the incapacity of these compounds to cross the outer membrane of these bacteria. The uptake of aminoglycosides through the outer membrane of Gram (−) bacteria is related to their ability to displace Ca^{2+} or Mg^{2+} from LPS, destroying the cross-bridging and destabilizing the outer membrane bilayer structure.[15] Thus, **10a** could be the sole derivative prepared which has the optimum lipophily/hydrophily balance to destabilize the outer membrane bilayer structure, probably by increasing the self-promoted uptake.

Fourth, the inhibition of the leucine incorporation in proteins of *P. aeruginosa* induced by **10a** was similar to that observed with polymyxin E (colistin), an antibacterial used in clinics to control chronic lung infections of multidrug resistant *P. aeruginosa* isolates in cystic fibrosis patients.[11] Recent AFM studies suggested that polymyxin E prevents *P. aeruginosa* proliferation by repressing cell division.[16] The exact mechanism of action of colistin is however not fully understood and could be more complex than originally assumed, i. e., binding to lipid A, release of LPS, disruption of the outer membrane, enhancement of self-promoted uptake, interaction with the cytoplasmic membrane, and increase of the permeability of the cell membrane. The formation of molecular contacts between the inner and outer membranes of the bacteria leading to lipid exchange resulting in loss of the compositional specificity of the membranes and osmotic instability,[17] together with increase in the rigidity of the bacterial cell wall and loss of LPS or surface proteins, could also contribute to the bactericidal effect of colistin. Further studies have to be done to know if the 3′,4′,6-2NM derivative **10a** has a similar mechanism of action.

In conclusion, interactions with the bacterial cell membrane are probably responsible for the excellent antimicrobial activity of **10a** in Gram (−) bacteria. Further studies are in progress with the aim to determine at the molecular level the binding modes and constants of our derivatives to bacterial lipids and to different rRNA sequences.

Experimental Section

Chemistry. General procedures. Procedure A was developed for the preparation of the 3′,6-di-*O*- and 3′,4′,6-tri-*O*-alkylated neamine derivatives from the tetratrityl derivative **12**: To a solution of **12**[8] (1 equiv) in DMF/THF (1/9) under argon were added NaH (60%, 4 equiv) and then, after 15 min at rt, the halide (3 equiv). The mixture was stirred for 24 h at rt and then DCM was added. The organic solution was washed with a saturated aqueous NH₄Cl solution, water, and then brine before drying over MgSO₄, filtration, and evaporation. The residue was chromatographed on alumina with cyclohexane/DCM to give the tetratrityl 3′,6-*O*- (**13**) and 3′,4′,6-*O*-arylmethylene (**14**) derivatives.

Procedure B was used for the removal of the trityl groups: The protected compound (**13** or **14**) was dissolved at 0 °C in DCM/TFA (1/1) in the presence of anisole (50 mg/mL). After 2 h, the solvents were evaporated. After addition of water and Et₂O, the aqueous phase was washed twice with Et₂O and evaporated. The residue was chromatographed on C18 reversed phase to lead to the TFA salts **7** and **10**, respectively.

Mono-2NM (2-5) and 4′,5-di2NM (8) Derivatives. 3′-Mono-*O*-[(2-naphthyl)methyl] neamine derivative **2**: prepared through the overdeprotection of **13a**, leading to removal of the 2NM group at position 6 according to procedure B at rt during 3 h. After purification on C18 reversed phase, the compound **7a** (3′,6-di2NM) and a mixture of **2** and **5** (6-mono2NM) were obtained. Both mono2NM neamine derivatives **2** and **5** were separated by chromatography on silica gel with EtOH/H₂O/NH₃ (90/10/20, v/v/v). After concentration of the fractions, the trifluoroacetate of **2** was converted to the chlorhydrate salt by ion exchange chromatography; 22% yield. ¹H NMR (400 MHz, D₂O) δ 7.95−8.02 (m, 4H, H-np), 7.58−7.65 (m, 3H, H-np), 5.95 (d, *J* = 3.6 Hz, 1H, H-1′), 5.17 (d, *J* = 11.0 Hz, 1H, CH₂-np), 5.01 (d, *J* = 11.0 Hz, 1H, CH₂-np), 4.16 (dd, *J* = 8.8 and 10.6 Hz, 1H, H-3′), 4.09 (ddd, *J* = 3.2, 7.1, and 10.0 Hz, 1H, H-5′), 4.00 (dd, *J* = 9.8 Hz, 1H, H-4), 3.75 (dd, *J* = 9.7 Hz, 1H, H-4′), 3.73 (dd, *J* = 9.3 Hz, 1H, H-5), 3.53−3.63 (m, 3H, H-3, H-6, H-2′), 3.49 (dd, *J* = 3.3 and 13.6 Hz, 1H, H-6′b), 3.37 (dd, *J* = 4.2 and 12.3 Hz, 1H, H-1), 3.32 (dd, *J* = 7.1 and 13.6 Hz, 1H, H-6′a), 2.52 (ddd, *J* = 4.2 and 12.6 Hz, 1H, H-2ax), 1.91 (ddd, *J* = 12.6 Hz, 1H, H-2eq). ¹³C NMR (100 MHz, D₂O) δ 136.3 and 134.5 (C-np), 130.1, 129.5, 129.3, 129.1, and 128.0 (CH-np), 97.6 (C-1′), 79.4 (C-4), 77.4 (C-3′), 76.6 (C-5 and CH₂-np), 74.1 (C-6), 72.7 and 71.3 (C-4′ and C-5′), 54.4 (C-2′), 51.2 and 50.0 (C-1 and C-3), 41.5 (C-6′), 29.9 (C-2). LRMS (ESI⁺) *m/z*: 463 [M + H] ⁺, 301, 163, 161, 141. HRMS (ESI⁺) *m/z*: [M + H]⁺ calculated 463.2557, found 463.2555, [M + Na]⁺ calculated 485.2376, found 485.2386; purity: 94% (HPLC).

4′-Mono-*O*- (**3**), 5-mono-*O*- (**4**), and 4′,5-di-*O*-[(2-naphthyl)-methyl] (**8**) neamine derivatives: synthesized according to the method previously described for the preparation of 4′- or 5-mono-*O*- and 4′,5-di-*O*-alkylated neamine derivatives.[8] To a solution of the *N*-tetratritylated 3′,6-diPMB neamine derivative[8] (1.0 g, 0.63 mmol) in 45 mL of dry DMF/THF mixture (1/9, v/v) under argon was added NaH (60%, 240 mg, 6 mmol), and after 15 min stirring at rt TBAI (237.7 mg, 0.63 mmol) and 2NMBr (975 mg, 4.41 mmol). The resulting mixture was stirred for 24 h at 70 °C, and then DCM was added. The organic phase was washed with an ammonium chloride aqueous saturated solution, water, and then brine before being dried over MgSO₄, filtrated, and concentrated under reduced pressure. The residue obtained was chromatographed on alumina gel with a gradient of cyclohexane/DCM from 80/20 to 50/50 to give the mono-*O*- and di-*O*-alkylated-tetra-*N*-tritylated neamine derivatives. Then the three alkylated compounds obtained were treated according to procedure B. **3**: 26% yield. ¹H NMR (400 MHz, CD₃OD) δ 7.81−7.88 (m, 4H, H-np), 7.44−7.52 (m, 3H, H-np), 5.95 (d, *J* = 3.8 Hz, 1H, H-1′), 5.16 (d, *J* = 11.5 Hz, 1H, CH₂-np), 4,82 (d, *J* = 11.5 Hz, 1H, CH₂-np), 4.22 (dd, *J* = 8.7 and 10.6 Hz, 1H, H-3′), 4.14 (ddd, *J* = 2.5 and 8.4 Hz, 1H, H-5′), 4.00 (dd, *J* = 9.8 Hz, 1H, H-4), 3,61 (dd, *J* = 9.2 Hz, 1H, H-5), 3.32−3.49 (m, 4H, H-3, H-6, H2′, H-4′), 3.27 (dd, *J* = 2.5 and 13.2 Hz, 1H, H-6′b), 3.18 (ddd, *J* = 2.0 and 12.0 Hz, 1H, H-1), 2.90 (dd, *J* = 9.4 and 13.2 Hz, 1H, H-6′a), 2.44 (ddd, *J* = 4.0 and 12.5 Hz, 1H, H-2eq), 1,99 (ddd, *J* = 12.0 Hz, 1H, H-2ax). ¹³C NMR (100 MHz, CD₃OD) δ 133.1−135.3 (3C-np), 125.7−128.0 (7CH-np), 95.7 (C-1′), 79.1 (C-4′), 77.9 (C-4), 75.8 (C-5), 74.6 (CH₂-np), 72.9 (C-6), 68.9 (C-5′ and C-3′), 54.2 (C-2′), 50.1 (C-1), 48.7 (C-3), 40.6 (C-6′), 28.4 (C-2). LRMS (DCI) *m/z*: 463 [M + H]⁺, 323 [M + H − 2NM]⁺. HRMS (ESI⁺) *m/z*: [M + H]⁺ calculated 463.2557, found 463.2538, [M + Na]⁺ calculated 485.2376, found 485.2385; purity: 93% (HPLC). **4**: 40% yield. ¹H NMR (400 MHz, D₂O) δ 7.81−7.89 (m, 4H, H-np),

7.47−7.50 (m, 3H, H-np), 5.81 (d, J = 3.6 Hz, 1H, H-1′), 5.00 (d, J = 11.2 Hz, 1H, CH$_2$-np), 4.79 (d, J = 11.2 Hz, 1H, CH$_2$-np), 4.03 (m, 1H, H-3′), 3.89−3.93 (m, 1H, H-5′), 3.74−3.79 (m, 1H, H-4), 3.56−3.60 (m, 1H, H-5), 3.43−3.50 (m, 2H, H-6, H-4′), 3.15−3.32 (m, 5H, H-2′, H-3, H-6′a, H-6′b, H-1), 2.84−2.90 (ddd, 1H, H-2eq), 2.26−2.31 (ddd, 1H, H-2ax). LRMS (ESI$^+$) m/z: 463 [M + H]$^+$, 323 [M + H − 2NM]$^+$. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 463.2557, found 463.2556. **8**: 10% yield. ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.82−7.94 (m, 8H, H-np), 7.45−7.58 (m, 6H, H-np), 5.86 (d, J = 3.0 Hz, 1H, H-1′), 5.26 (d, J = 11.0 Hz, 1H, CH$_2$-np), 4.97 (d, J = 11.0 Hz, 1H, CH$_2$-np), 4.80−4.95 (m, 2H, CH$_2$-np), 4.25−4.33 (m, 2H, H-3′, H-5′), 4.18 (t, J = 9.4 Hz, 1H, H-4), 3.78 (t, J = 8.8 Hz, 1H, H-5), 3.71 (t, J = 9.5 Hz, 1H, H-6), 3.52 (m br, 1H, H-3), 3.46 (t, J = 6.0 Hz, 1H, H-4′), 3.41 (dd, J = 3.0 and 7.0 Hz, 1H, H-2′), 3.10−3.32 (m, 3H, H-1, H-6′b, H-6′a), 2.45 (ddd, J = 4.0 and 12.8 Hz 1H, H-2eq), 1.98 (ddd, J = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, CD$_3$OD) δ 132.0−137.0 (6C-np), 127−130 (14CH-np), 93.9 (C-1′), 84.7 (C-5), 78.1 (C-4′), 77.5 (C-4), 76.1 (CH$_2$-np), 75.1 (C-6 and CH$_2$-np), 73.4 (C-5′), 69.2 (C-3′), 54.38 (C-2′), 51.8 (C-3), 50.7 (C-1), 41.0 (C-6′), 29.7 (C-2). LRMS (DCI) m/z: 603 [M + H]$^+$, 463 [M + H − 2NM]$^+$; purity: 98% (HPLC).

6-Mono-*O*-[(2-naphthyl)methyl] neamine derivative 5: prepared according to the method previously used for the regioselective alkylation of neamine in the position 6.[9] To a solution of **12**[8] (502 mg) in 6 mL of DMF/THF (1/1, v/v) under argon was first added NaH (60%, 170 mg, 10 equiv) and after 15 min stirring at rt the 2-NMBr (130 mg, 5 equiv). The resulting mixture was stirred for 4 h at rt, and then DCM was added. The organic phase was washed with an ammonium chloride aqueous saturated solution, water, and then brine before being dried over MgSO$_4$, filtrated, and concentrated under reduced pressure. The residue obtained was chromatographed on alumina gel with a gradient of DCM/methanol from 100/0 to 99.8/0.2 to give the protected compound in 12% yield. LRMS (MALDI, DHB) m/z: 1454 [M + Na]$^+$, 1430 [M + H]$^+$, 1211 [M − Tr + Na]$^+$, 1187 [M − Tr + H]$^+$. Then procedure B was applied for obtaining **5**: 82% yield. ^{1}H NMR (400 MHz, D$_2$O) δ 7.86−7.91 (m, 2H, H-np), 7.48−7.53 (m, 2H, H-np), 5.89 (d, J = 3.6 Hz, 1H, H-1′), 5.04 (d, J = 11.2 Hz, 1H, CH$_2$-np), 4.88 (d, J = 11.2 Hz, 1H, CH$_2$-np), 3.88−3.98 (m, 3H, H-3′, H-5′, H-4), 3.85 (dd, J = 8.8 and 9.2 Hz, 1H, H-5), 3.30−3.49 (m, 5H, H-3, H-4′, H-2′, H-6′b, H-1), 3.22 (dd, J = 6.8 and 13.6 Hz, 1H, H-6′a), 2.42 (ddd, J = 4.0 and 12.4 Hz, 1H, H-2eq), 1.83 (ddd, J = 12.4 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D$_2$O) δ 134.4−132.9 (3C-np), 128.6−126.4 (7CH-np), 96.0 (C-1′), 79.9 (C-6), 77.5 (C-4), 75.8 (C-5), 75.2 (CH$_2$-np), 70.6 (C-4′), 69.3 (C-5′), 68.2 (C-3′), 53.5 (C-2′), 48.9 (C-1), 48.4 (C-3), 40.1 (C-6′), 28.2 (C-2). LRMS (FAB$^+$, NBA) m/z: 463 [M + H]$^+$, 303, 161, 141. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 463.2557, found 463.2545; purity: 95% (HPLC).

3′,4′-Di-*O*-[(2-naphthyl)methyl] neamine derivative 6: prepared from **14a** through the selective removal of the 2-NM group at the position 6 according to procedure B at rt during 3 h. 51% yield. ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.30−7.85 (m, 14H, H-np), 6.03 (d, J = 3.6 Hz, 1H, H-1′), 4.80−5.04 (m, 4H, 2CH$_2$-np), 4.30−4.40 (m, 2H, H-3′, H-5′), 4.08 (dd, J = 9.7 Hz, 1H, H-4), 3.58−3.67 (m, 3H, H-5, H-4′, H-2′), 3.39−3.53 (m, 2H, H-6, H-3), 3.34 (dd, J = 3.5 and 13.5 Hz, 1H, H-6′b), 3.21 (ddd, J = 4.2 and 12.5 Hz, 1H, H-1), 3.10 (dd, J = 9.8 and 13.3 Hz, 1H, H-6′a), 2.47 (ddd, J = 4.0 and 12.4 Hz, 1H, H-2eq), 1.30 (ddd, J = 12.5 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D$_2$O) δ 132.7−134.4 (6C-np), 125.3−128.4 (14CH-np), 95.4 (C-1′), 78.2 (C-4′), 77.7 (C-4), 75.7 (C-3′), 75.1 (C-5), 74.9 (2CH$_2$-np), 72.3 (C-6), 69.7 (C-5′), 52.6 (C-2′), 49.6 (C-1), 48.4 (C-3), 39.8 (C-6′), 28.2 (C-2). LRMS (ESI$^+$) m/z: 603 [M + H]$^+$, 463, 441, 301, 141. HRMS (ESI$^+$) m/z: [M + Na]$^+$ calculated 625.3002, found 625.3024; purity: 96% (HPLC).

3′,6-Di- (7a−d) and 3′,4′,6-Trialkylated (10a−d) Neamine Derivatives. 3′,6-Di-*O*- (**7a**) and 3′,4′,6-tri-*O*-[(2-naphthyl)methyl] (**10a**) derivatives: **13a** and **14a** were prepared in 40% and 38% yields, respectively, according to procedure A from **12** (2.0 g), DMF/THF (1/9, 20 mL), NaH (217 mg), 2NMBr (857 mg), and after chromatography with cyclohexane/DCM (50/50). **13a**: LRMS (FAB$^+$, NBA) m/z: 1594 [M + Na]$^+$. **14a**: LRMS (FAB$^+$, NBA) m/z: 1735 [M + Na]$^+$. **13a** (602 mg, 0.39 mmol) and **14a** (121 mg, 0.07 mmol), respectively, were treated according to procedure B. **7a** and **10a** were isolated as chlorhydrate salts after ion exchange chromatography in 70 and 65% yields, respectively. HPLC analysis showed 94 and 96% purity for **7a** and **10a**, respectively. **7a**: 70% yield. ^{1}H NMR (400 MHz, D$_2$O) δ 7.84−7.89 (m, 8H, H-np), 7.47−7.50 (m, 6H, H-np), 5.86 (d, J = 3.6 Hz, 1H, H-1′), 5.05 (d, J = 12.0 Hz, 1H, CH$_2$-np), 5.02 (d, J = 12.0 Hz, 1H, CH$_2$-np), 4.88 (d, J = 12.0 Hz, 1H, CH$_2$-np), 4.85 (d, J = 12.0 Hz, 1H, CH$_2$-np), 4.04 (dd, J = 8.5 and 10.4 Hz, 1H, H-3′), 3.96 (m, 1H, H-5′), 3.91 (dd, J = 10.0 Hz, 1H, H-4), 3.82 (dd, J = 9.2 Hz, 1H, H-5), 3.65 (dd, J = 9.2 Hz, 1H, H-4′), 3.58 (dd, J = 9.2 Hz, 1H, H-6), 3.45 (dd, J = 3.6 and 10.4 Hz, 1H, H-2′), 3.43−3.28 (m, 3H, H-3, H-6′b, H-1), 3.22 (dd, J = 9.6 and 12.8 Hz, 1H, H-6′a), 2.42 (ddd, J = 4.0 and 12.4 Hz, 1H, H-2eq), 2.01 (ddd, J = 12.4 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, CD$_3$OD) δ 133.1−135.6 (6C-np), 125.6−127.7 (14CH-np), 94.4 (C-1′), 80.3 (C-6), 76.6 (C-4), 75.9 and 75.8 (C-5 and C-3′), 74.9 (2CH$_2$-np), 72.2 (C-4′), 70.4 (C-5′), 53.1 (C-2′), 49.3 (C-1), 48.9 (C-3), 40.4 (C-6′), 28.3 (C-2). LRMS (DCI$^+$) m/z: 603 [M + H]$^+$, 463, 303, 141. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 603.3183, found 603.3186, [M + Na]$^+$ calculated 625.3002, found 625.3006; purity: 94% (HPLC). **10a**: 65% yield. ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.39−7.97 (m, 21H, H-np), 6.03 (d, J = 3.6 Hz, 1H, H-1′), 4.94−5.29 (m, 6H, CH$_2$-np), 3.57−3.59 (m, 2H, H-3′, H-5′), 4.20 (dd, J = 9.6 Hz, 1H, H-4), 4.08 (dd, J = 9.2 Hz, 1H, H-5), 3.65−3.74 (m, 2H, H-6, H-4′), 3.49−3.56 (m, 2H, H-3, H-2′), 3.35−3.42 (m, 2H, H-6′b, H-1), 3.17 (dd, J = 9.6 and 13.2 Hz, 1H, H-6′a), 2.46 (ddd, J = 4.0 and 12.4 Hz, 1H, H-2eq), 2.01 (ddd, J = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, CD$_3$OD) δ 133.1−135.4 (9C-np), 125.3−127.9 (21CH-np), 95.2 (C-1′), 80.5 (C-6), 78.9 and 78.5 (C-4 and C-4′), 77.5 (C-3′), 76.3 (C-5), 74.7 and 74.4 (2CH$_2$-np), 69.7 (C-5′), 53.4 (C-2′), 49.5 (C-1), 48.6 (C-3), 40.3 (C-6′), 29.5 (C-2). LRMS (DCI$^+$) m/z: 743 [M + H]$^+$, 603 [M + H − 2NM]$^+$, 441, 303, 141. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 743.3809, found 743.3810, [M + Na]$^+$ calculated 765.3628, found 765.3628; purity: 96% (HPLC).

3′,6-Di-*O*-benzyl (7b) and 3′,4′,6-tri-*O*-benzyl (10b) derivatives: **13b** and **14b** were obtained respectively in 35% and 45% yields according to procedure A with DMF/THF (1/9, v/v) as solvents and benzyl bromide as alkylating agent. After deprotection, the trifluoroacetate salts of **7b** and **10b** were converted to the chlorydrate salts as **7a** and **10a**. **7b**: 98% yield. ^{1}H NMR (400 MHz, D$_2$O) δ 7.30−7.40 (m, 10H, H-Bn), 5.86 (d, J = 3.6 Hz, 1H, H-1′), 4.89 (d, J = 11.0 Hz, 1H, CH$_2$-Bn), 4.87 (d, J = 11.0 Hz, 1H, CH$_2$-Bn), 4.65−4.73 (m, 2H, CH$_2$-Bn), 4.00 (dd, J = 8.8 and 10.4 Hz, 1H, H-3′), 3.91−3.98 (m, 2H, H-5′, H-4), 3.81 (dd, J = 9.2 Hz, 1H, H-5), 3.61 (dd, J = 9.2 Hz, 1H, H-4′), 3.57 (dd, J = 9.2 and 10.4 Hz, 1H, H-6), 3.35−3.46 (m, 3H, H-2′, H-6′b, H-3), 3.29 (m, 1H, H-1), 3.21 (dd, J = 7.2 and 13.6 Hz, 1H, H-6′a), 2.39 (ddd, J = 4.4 and 12.4 Hz, 1H, H-2eq), 1.80 (ddd, J = 12.4 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D$_2$O) δ 137.0 and 136.7 (2C-Bn), 128.7−128.9 (10CH-Bn), 95.7 (C-1′), 79.8 (C-6), 77.3 (C-4), 75.7 (C-5 and C-3′), 75.1 and 75.2 (2CH$_2$-Bn), 71.0 (C-4′), 69.8 (C-5′), 52.6 (C-2′), 48.8 (C-1), 48.4 (C-3), 39.9 (C-6′), 28.2 (C-2). LRMS (DCI$^+$) m/z: 503 [M + H]$^+$, 295, 253. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 503.2870, found 503.2871, [M + Na]$^+$ calculated 525.2689, found 525.2689, [M + K]$^+$ calculated 541.2428, found 541.2451; purity: 99% (HPLC). **10b**: 99% yield. ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.31−7.49 (m, 15H, H-Bn), 6.11 (d, J = 3.6 Hz, 1H, H-1′), 5.11 (d, J = 10.9 Hz, 1H, CH$_2$-Bn), 4.98 (d, J = 11.3 Hz, 1H, CH$_2$-Bn), 4.93 (d, J = 11.3 Hz, 1H, CH$_2$-Bn), 4.82 (m, 2H, CH$_2$-Bn), 4.71 (d, J = 11.3 Hz,

1H, CH$_2$-Bn), 4.32−4.44 (m, 3H, H-3′, H-5′, H-4), 3.98 (dd, J = 9.2 Hz, 1H, H-5), 3.67 (dd, J = 10.0 Hz, 1H, H-6), 3.55−3.63 (m, 3H, H-3, H-2′, H-4′), 3.34−3.42 (m, 2H, H-6′b, H-1), 3.19 (dd, J = 9.8 and 13.3 Hz, 1H, H-6′a), 2.48 (ddd, J = 4.4 and 12.3 Hz, 1H, H-2eq), 2.09 (ddd, J = 12.4 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, CD$_3$OD) δ 137.3 and 137.2 (3C-Bn), 127.4−128.2 (15CH-Bn), 94.1 (C-1′), 80.4 (C-6), 78.2 (C-4′), 77.0 (C-4), 76.3 (C-5), 76.1 (C-3′), 74.8, 74.5, and 74.42 (CH$_2$-Bn), 70.1 (C-5′), 52.7 (C-2′), 49.3 (C-1), 48.7 (C-3), 40.0 (C-6′), 28.6 (C-2). LRMS (DCI$^+$) m/z: 593 [M + H]$^+$, 341, 253. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 593.3339, found 593.3341, [M + Na]$^+$ calculated 615.3158, found 615.3154; purity: 99% (HPLC).

3′,6-Di-*O*-[(2-pyridyl)methyl] (**7c**) and 3′,4′,6-tri-*O*-[(2-pyri-dyl)methyl] (**10c**) neamine derivatives: prepared as **7a** and **10a** using 2-(chloromethyl)pyridine (free base) as alkylating agent and DMF/THF (1/9, v/v) as solvents. The intermediate pro-tected compounds **13c** and **14c** were obtained with 30% and 15% yields respectively after chromatography with a gradient of cyclohexane/DCM from 50/50 to 20/80. **13c**: LRMS (MALDI, DHB) m/z: 1497 [M + Na]$^+$, 1475 [M + H]$^+$, 1255 [M − Tr + Na]$^+$, 1231 [M − Tr + H]$^+$. **14c**: LRMS (MALDI, DHB) m/z: 1588 [M + Na]$^+$, 1566 [M + H]$^+$, 1346 [M − Tr + Na]$^+$, 1323 [M − Tr + H]$^+$. **13c** and **14c** then were treated according to procedure B for obtaining **7c** and **10c**, respectively. **7c**: 80% yield. ^{1}H NMR (400 MHz, D$_2$O) δ 8.44 (d, J = 4.8 Hz, 1H, H-py), 8.30 (m, 2H, H-py), 7.85 (m, 1H, H-py), 7.74 (m, 1H, H-py), 7.23−7.41 (m, 7H, H-py), 5.88 (d, J = 4.0 Hz, 1H, H-1′), 4.98 (d, J = 13.6 Hz, 1H, CH$_2$-py), 4.86 (d, J = 13.2 Hz, 1H, CH$_2$-py), 4.65−4.81 (m, 4H, CH$_2$-py), 4.22 (dd, J = 8.4 and 10.0 Hz, 1H, H-3′), 4.11 (ddd, J = 3.2 and 8.4 Hz, 1H, H-5′), 3.91 (dd, J = 9.6 Hz, 1H, H-4), 3.85 (dd, J = 8.8 and 9.2 Hz, 1H, H-5), 3.66 (dd, J = 8.8 Hz, 1H, H-4′), 3.63 (dd, J = 9.2 and 10.0 Hz, 1H, H-6), 3.57 (dd, J = 3.6 and 10.4 Hz, 1H, H-2′), 3.28−3.46 (m, 3H, H-3, H-1, H-6′), 3.16 (dd, J = 8.4 and 13.2 Hz, 1H, H-6′a), 2.42 (ddd, J = 4.0 and 8.4 Hz, 1H, H-2eq), 1.82 (ddd, J = 8.4 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D$_2$O) δ 154.9−154.6 (3C-py), 147.4−122.7 (12CH-py), 95.6 (C-1′), 81.9 (C-6), 79.0 (C-4′), 77.5 and 77.0 (C-4 and C-3′), 75.3 (C-5), 74.0 and 73.5 (2CH$_2$-py), 69.1 (C-5′), 52.7 (C-2′), 48.9 (C-1), 48.3 (C-3), 39.9 (C-6′), 28.4 (C-2). LRMS (ESI$^+$) m/z: 618 [M + Na]$^+$, 596 [M + H]$^+$, 345, 254. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 596.3197, found 596.3183, [M + Na]$^+$ calculated 618.3016, found 618.3028; purity: 99% (HPLC). **10c**: 20% yield. ^{1}H NMR (400 MHz, D$_2$O) δ 8.62 (dd, J = 1.2 and 6.4 Hz, 2H, H-py), 8.43 (ddd, J = 1.2 and 8.0 Hz, 2H, H-py), 7.83−7.89 (m, 4H, H-py), 5.89 (d, J = 3.6 Hz, 1H, H-1′), 5.30 (d, J = 15.2 Hz, 2H, CH$_2$-py), 5.18 (d, J = 14.0 Hz, 1H, CH$_2$-py), 5.13 (d, J = 14.8 Hz, 1H, CH$_2$-py), 4.15 (dd, J = 8.8 and 10.8 Hz, 1H, H-3′), 3.89−4.01 (m, 3H, H-5′, H-4, H-5), 3.76 (dd, J = 8.8 and 10.0 Hz, 1H, H-6), 3.68 (dd, J = 8.8 and 9.6 Hz, 1H, H-4′), 3.60 (dd, J = 3.6 and 10.8 Hz, 1H, H-2′), 3.45−3.54 (m, 2H, H-3, H-1), 3.37 (dd, J = 2.4 and 13.6 Hz, 1H, H-6′b), 3.18 (dd, J = 6.8 and 13.6 Hz, 1H, H-6′a), 2.48 (ddd, J = 4.0 and 12.8 Hz, 1H, H-2eq), 1.90 (ddd, J = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D$_2$O) δ 152.0 and 151.8 (C-py), 146.5−124.8 (8CH-py), 95.9 (C-1′), 81.3 (C-6), 77.3 (C-4 et C-3′), 75.4 (C-5), 70.8 (C-4′), 70.0 (2CH$_2$-py), 69.5 (C-5′), 52.4 (C-2′), 48.6 (C-1), 48.2 (C-3), 39.8 (C-6′), 28.2 (C-2). LRMS (FAB$^+$, NBA) m/z: 506 [M + H]$^+$, 414, 381, 254. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 505.2775, found 505.2769, [M + Na]$^+$ calculated 527.2594, found 527.2589; purity: 85% (HPLC).

3′,6-Di-*O*-[(2-quinolyl)methyl] (**7d**) and 3′,4′,6-tri-*O*-[(2-quinolyl)methyl] (**10d**) neamine derivatives: prepared as **7a** and **10a** using 2-(chloromethyl)quinoline (free base) as alkylat-ing agent and DMF/THF (1/9, v/v) as solvents. The protected compounds **13d** and **14d** were obtained with 52% and 11% yields, respectively, after chromatography with a gradient of cyclohexane/DCM from 50/50 to 30/70. **13d**: LRMS (MALDI, DHB) m/z: 1596 [M + Na]$^+$, 1574 [M + H]$^+$, 1353 [M − Tr + Na]$^+$, 1331 [M − Tr + H]$^+$. **14d**: LRMS (MALDI, DHB)

m/z: 1738 [M + Na]$^+$, 1716 [M + H]$^+$, 1496 [M − Tr + Na]$^+$, 1472 [M − Tr + H]$^+$. **13d** and **14d** then were treated according to procedure B for obtaining **7d** and **10d**, respectively. **7d**: 82% yield. ^{1}H NMR (400 MHz, D$_2$O) δ 8.67 (d, J = 8.4 Hz, 1H, H-qui), 8.60 (d, J = 8.8 Hz, 1H, H-qui), 7.66−8.06 (m, 9H, H-qui), 7.63 (d, J = 8.4 Hz, 1H, H-qui), 5.93 (d, J = 3.6 Hz, 1H, H-1′), 5.37 (d, J = 15.2 Hz, 1H, CH$_2$-qui), 5.33 (d, J = 15.2 Hz, 1H, CH$_2$-qui), 5.25 (d, J = 15.2 Hz, 1H, CH$_2$-qui), 5.21 (d, J = 15.2 Hz, 1H, CH$_2$-qui), 4.17 (dd, J = 8.8 and 10.8 Hz, 1H, H-3′), 3.92−4.03 (m, 3H, H-5′, H-4, H-5), 3.76 (dd, J = 8.8 and 10.4 Hz, 1H, H-6), 3.72 (dd, J = 8.8 Hz, 1H, H-4′), 3.64 (dd, J = 3.6 and 10.8 Hz, 1H, H-2′), 3.46−3.57 (m, 2H, H-3, H-1), 3.38 (dd, J = 3.6 and 13.6 Hz, 1H, H-6′b), 3.20 (dd, J = 6.8 and 13.6 Hz, 1H, H-6′a), 2.50 (ddd, J = 4.4 and 12.8 Hz, 1H, H-2eq), 1.89 (ddd, J = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D$_2$O) δ 156.4−127.9 (6C-qui), 143.6−119.7 (12CH-qui), 96.1 (C-1′), 81.9 (C-6), 77.7 and 77.6 (C-4 and C-3′), 75.4 (C-5), 72.6 and 72.2 (2CH$_2$-qui), 70.8 (C-4′), 69.5 (C-5′), 52.6 (C-2′), 48.8 (C-1), 48.3 (C-3), 39.8 (C-6′), 28.3 (C-2). LRMS (FAB$^+$, NBA) m/z: 605 [M + H]$^+$. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 605.3088, found 605.3092; purity: 94% (HPLC). **10d**: 34% yield. ^{1}H NMR (400 MHz, D$_2$O) δ 8.34 (d, J = 8.4 Hz, 1H, H-qui), 7.10−7.92 (m, 17H, H-qui), 5.95 (d, J = 3.2 Hz, 1H, H-1′), 5.19 (d, J = 14.4 Hz, 1H, CH$_2$-qui), 5.08 (d, J = 14.8 Hz, 1H, CH$_2$-qui), 4.96 (d, J = 15.2 Hz, 1H, CH$_2$-qui), 4.89 (d, J = 14.0 Hz, 1H, CH$_2$-qui), 4.70 (d, J = 14.8 Hz, 1H, CH$_2$-qui), 4.52 (d, J = 14.4 Hz, 1H, CH$_2$-qui), 4.29 (dd, J = 9.2 and 10.0 Hz, 1H, H-3′), 4.20 (ddd, J = 2.4 and 8.4 Hz, 1H, H-5′), 3.98 (dd, J = 9.2 and 10.0 Hz, 1H, H-4), 3.93 (dd, J = 9.2 Hz, 1H, H-5), 3.77 (dd, J = 9.2 Hz, 1H, H-4′), 3.70 (dd, J = 9.2 and 10.0 Hz, 1H, H-6), 3.65 (dd, J = 3.2 and 10.4 Hz, 1H, H-2′), 3.47−3.55 (m, 2H, H-3, H-1), 3.44 (dd, J = 2.8 and 13.6 Hz, 1H, H-6′b), 3.28 (dd, J = 8.4 and 13.6 Hz, 1H, H-6′a), 2.48 (ddd, J = 4.0 and 12.8 Hz, 1H, H-2eq), 1.88 (ddd, J = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D$_2$O) δ 157.1−142.7 (9C-qui), 140.5−118.5 (18CH-qui), 95.9 (C-1′), 82.1 (C-6), 80.1 (C-4′), 77.5 and 77.1 (C-4 and C-3′), 75.5 (C-5), 73.7 (CH$_2$-qui), 69.3 (C-5′), 53.0 (C-2′), 49.0 (C-1), 48.4 (C-3), 40.0 (C-6′), 28.4 (C-2). LRMS (FAB$^+$, NBA) m/z: 747 [M + H]$^+$. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 748.3714, found 748.3743, [M + Na]$^+$ calculated 770.3534, found 770.3563; purity: 90% (HPLC).

4′,6-Di-*O*-[(2-naphthyl)methyl] neamine derivative **9**: **12** (1 g, 1 equiv, 0.76 mmol) was dissolved in 25 mL of anhydrous toluene under argon. NaH (60%, 125 mg, 4 equiv, 3.12 mmol) was added to the solution and then, after 15 min stirring, *p*-methoxybenzylchloride (0.5 mL, 4 equiv, 2.76 mmol) was added. Twenty-two days later, the reaction was stopped in adding ethanol and the reaction mixture was diluted with ethyl acetate. The organic layer was washed with water, Na$_2$SO$_3$, brine, and dried over MgSO$_4$. The solvent was removed under reduce pressure, and the residue was chromatographed on alumina gel with a gradient of cyclohexane to toluene to ethyl acetate. The *N*-tetratritylated-3′-monoPMB neamine deriva-tive was obtained in 55% yield. LRMS (MALDI, DHB) m/z: 1435 [M + Na]$^+$, 1412 [M + H]$^+$, 1169 [M − Tr + H]$^+$, 927 [M − 2Tr + H]$^+$.

Under anhydrous conditions, NaH (60%, 33 mg, 3 equiv, 0.83 mmol) was added to a solution of this compound (390 mg, 1 equiv, 0.28 mmol) in 15 mL of DMF. The mixture was stirred for 15 min at 0 °C in an ice bath, and then 2NMBr (152 mg, 2.5 equiv, 0.69 mmol) was added. The mixture was stirred for 30 min at 0 °C and then overnight at rt. Another portion of 2NMBr (152 mg) was added, and the mixture was stirred for 12 h more. The completion of the reaction was monitored by TLC (toluene/ ethyl acetate: 9/1). The solvent of the mixture was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate and washed twice with water and brine. The organic layer was dried over MgSO$_4$ and concentrated under reduced pressure. The product was directly treated according to procedure B for obtaining **9** as a trifluoroacetic acid salt: 15%

yield. ^{1}H NMR (400 MHz, D$_2$O) δ 7.90−7.50 (m, 14H, H-np), 6.01 (d, J = 3.6 Hz,1H, H-1′), 5.20 (m, 2H, CH$_2$-np), 4.90 (m, 2H, CH$_2$-np), 4.27 (dd, J = 8 and 12 Hz,1H, H-3′), 4.18 (m, 1H, H-5′), 4.11 (dd, J = 10 Hz,1H, H-4), 3.91 (dd, J = 10 Hz, 1H, H-5), 3.64 (dd, J = 8 Hz, 1H, H-6), 3.28−3.46 (m, 5H, H-1, H-3, H-2′, H-4′, H-6′b), 2.98 (dd, J = 8 and 12 Hz, 1H, H-6′a), 2.50 (m, 1H, H-2b), 2.08 (m, 1H, H-2a). ^{13}C NMR (100 MHz, D$_2$O) δ 132.0−135.0 (6C-np), 129.9−127.3 (14CH-np), 97.2 (C-1′), 82.0 (C-6), 80.5 (C-4′), 79.5 (C-4), 78.0 (C-5), 76.4 (CH$_2$-np), 76.1 (CH$_2$-np), 70.6 (C-3′), 70.3 (C-5′), 55.6 (C-2′), 50.8 (C-1), 50,2 (C-3), 42.1 (C-6′), 30.0 (C-2). HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 603.3183, found 603.3199, [M + Na]$^+$ calculated 625.3002, found 625.3005, [M + K]$^+$ calculated 641.2741, found 641.2726.

3′,4′,5,6-Tetra-*O*-[(2-naphthyl)methyl] neamine derivative **11**: prepared according to procedure A from 300 mg of **12** in 7 mL of DMF using a large excess of NaH (10 equiv) and 2NMBr (7 equiv). The protected derivative was obtained in 66% yield. LRMS (MALDI, DHB) m/z: 1874 [M + Na]$^+$, 1852 [M + H]$^+$. The deprotection was achieved according to procedure B for obtaining **11**: 60% yield. ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.26−7.90 (m, 27H, H-np), 6.89 (d, 1H, J = 6.8 Hz, H-np), 6.67 (d, J = 2.0 Hz, 1H, H-1′), 4.80−5.15 (m, 4H, 2CH$_2$-np), 4.65−4.80 (m, 2H, CH$_2$-np), 4.62 (m, 1H, J = 10.4 Hz, H-5′), 4.39 (m, 1H, H-4), 4.21−4.31 (m, 2H, CH$_2$-np), 4.04 (m, 1H, H-3′), 3.85−3.93 (m, 2H, H-6, H-5), 3.40−3.70 (m, 5H, H-1, H-3, H-2′, H-4′, H-6′b), 3.04 (m, J = 12.4 Hz, 1H, H-6′a), 2.55 (m, J = 12.4 Hz, 1H, H-2eq), 2.11 (m, J = 12.4 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, CD$_3$OD) δ 133.0−135.1 (4C-np), 124.4−128.2 (28CH-np), 92.1 (C-1′), 83.4 (C-5), 80.3 (C-6), 79.0 (C-4), 75.4 and 75.1 (2CH$_2$-np), 74.8 (C-5′), 72.8 (C-3′), 72.5 and 72.1 (2CH$_2$-np), 71.8 (C-4′), 49.5 and 48.9 (C-1, C-3 and C-2′), 38.3 (C-6′), 28.3 (C-2). LRMS (DCI$^+$) m/z: 743 [M + H]$^+$, 603 [M + H − 2NM]$^+$, 441, 303, 141. HRMS (ESI$^+$) m/z: [M + H]$^+$ calculated 743.3809, found 743.3810, [M + Na]$^+$ calculated 765.3628, found 765.3628.

Antimicrobial Effects. The minimal inhibitory concentrations (MICs) were determined by a geometric microdilution method according to the recommendations of the CLSI norms for Gram negative strains (*E. coli* (ATCC 25922, Ec06AB003 (Arm), EcPAZ505H8101 and EcL58058.1), *P. aeruginosa* (ATCC 27853, PA02, PA03, PAO1, PA21, PA22, PA405, PA406 and Psa.F03), *A. lwoffi* (ATCC 17925 and Al.88−483), *C. amalonaticus* Ca06AB0010 (Arm), *E. aerogenes* 06AB008 (Arm)), and *S. aureus* strains (ATCC 33592 HA-MRSA and VRSA VRS-2).[18]

The method was slightly modified for *S. aureus* (ATCC 25923, *S. aureus* SA-1199B (harboring resistance to fluoroquinolones through overexpression of the NorA efflux pump), *S.aureus* MsrA (resistant to 14- and 15-membered macrolides, harboring the multicopies plasmid pUL 5054 coding for an efflux pump), *S. aureus* APH2″-AAC6′ (aminoglycoside-6′-*N*-acetyltransferase/2″-O-phosphoryltransferase), *S. aureus* APH3′ (aminoglycoside-3′-*O*-phosphoryltransferase), and *S. aureus* ANT4′ (aminoglycoside-4′-*O*-phosphoryltransferase). Briefly, the plates were incubated at 37 °C, and bacterial growth was monitored at 650 nm after 1, 4, 7, and 24 h of growth. Ampicillin (16 mg/L) was used as a positive control and 2 μL of DMSO as a negative control. The extract was considered as very active if there was no bacterial growth after 24 h incubation, as active if bacterial growth was less than 10% of the negative control and inactive if bacterial growth was more than 10% of the negative control.

Alteration of Protein Synthesis. Overnight cultures of *P. aeruginosa* ATCC 27853 were diluted in cation adjusted Muller−Hinton broth (CA-MHB) media and allowed to grow to the exponential phase (optical density at 620 nm, 0.5−0.8). Cultures were then centrifuged at 4000 rpm at 4 °C and washed two times with CA-MHB medium. Culture samples of 1.5 mL were incubated with 0.1, 0.25, 0.5, 1, 2.5, 5, and 10-fold MICs of

10a, neamine **1**, neomycin B, polymyxin E, and aztreonam at 37 °C during 1 h under shaking (150 rpm). Samples were then centrifuged at 4000 rpm at 4 °C, washed 1 time with CA-MHB medium, and resuspended in CA-MHB medium containing 2 μCi/mL of L-[4,5-^{3}H]leucine. Samples were incubated during 150 min at 37 °C under shaking (150 rpm) and then centrifuged at 4000 rpm at 4 °C and washed two times with ice-cold PBS buffer. An aliquot of 250 μL of each sample was placed in a 20 mL scintillation vial with 10 mL of Perkin-Elmer Ultima Gold liquid scintillation cocktail and total counts were obtained in a Tri-Carb 2800TR Perkin-Elmer liquid scintillation analyzer. Another 500 μL aliquot was treated with ice-cold 10% trichloroacetic acid for 10 min and frozen overnight. After centrifugation at 4000 rpm at 4 °C, 250 μL of the supernatant was analyzed to access to soluble counts. The incorporation of L-[4,5-^{3}H]leucine was obtained by the subtraction of the soluble counts to the total counts divided by the protein concentration obtained by the Lowry method.[10]

Isothermal Titration Calorimetry (ITC). ITC measurements were performed at 25 °C on a MicroCal VP-ITC (MicroCal, Northampton, MA). The buffer contained 25 mM potassium acetate, 2 mM magnesium acetate, 20 mM sodium cacodylate, pH 7.0. Forty 5 μL aliquots of 200 μM solution of drugs were injected into a 2 μM of a model bacterial ribosomal A-site (1.42 mL sample cell).[12] The duration of each injection was 10 s, and the delay between injections was 240 s. ITC titration curves were analyzed using the software Origin (OriginLab, Northampton, MA). A control experiment was performed with neomycin B.[13]

Supporting Information Available: HPLC profiles, ^{1}H and ^{13}C NMR spectra of the neamine derivatives **6**, **7a**, and **10a** active as antimicrobial agents, table of purity determined for all evaluated derivatives and ITC profiles at 25 °C for the titration of compounds **7a** and **10a** into a solution of a model bacterial ribosomal A site. This material is available free of charge via the Internet at http://pubs.acs.org.

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